Gaussian distributions after every time step (*dt=10ps*). Unidirectional particle fluxes were determined by counting particles that crossed the channel (particle gradient *4* to *0*).
Uncharged particle flux was not affected by the pore's mouth shape. When $e=-2e^-$, particle flux depended on pore's shape ($C_{1to2}=1.6\pm0.04x10^6$ *particles/sec/channel [p/s/ch]*; $C_{2to1}=0.75\pm0.03x10^6$ *p/s/ch*). A negative charged ring reduced/blocked particle flux and pore-particle interactions. A positive charged ring doubled particle flux for C_{2to1} only, highlighting the mechanism's complexity.
Particle number at steady-state (SS) during bidirectional fluxes was calculated after placing 8 particles on either side. The average number of particles per cell remained close to 4 at SS and did not vary significantly (*<6%;n=15*) over *6μs;* the time to reach steady state for C_{1to2} was smaller (*1.5μs* and *2.1μs* for C_{2to1}). Our mathematical model explains preferential directional fluxes by differences in pore mouth's shape. Identical number of particles at SS indicates thermodynamics compliance.

503-Pos
Propagation Mechanisms of Calcium Waves Arising During Arterial Vasomotion
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Vasomotion consists in cyclic arterial diameter variations induced by synchronous contractions and relaxations of smooth muscle cells (SMCs) present in the arterial wall. These contractions have been shown to be due to an increase in the intracellular cytosolic calcium concentration. However, the arteries do not contract simultaneously on macroscopic distances and a propagation of the diameter variations can be observed. Our aim was to investigate this propagation. We stimulated endothelium denuded rat mesenteric arterial strips with the vasoconstrictor, phenylephrine (PE) to obtain vasomotion and observed that the contraction waves are linked to intercellular calcium waves through the SMCs. A velocity of about 100 μm/s was measured for the two kinds of waves which could propagate either from the distal to the proximal side of the artery or in the opposite direction. To investigate the calcium wave propagation mechanisms, we used a method allowing a PE stimulation of a small area of the strip. No calcium propagation could be induced by this local stimulation when the strip was in its resting state. However, if a low PE concentration was added to the whole strip, local PE stimulations induced calcium waves spreading over finite distances. The calcium wave velocity induced by local stimulation was identical to the velocity observed during vasomotion. This suggests that the propagation mechanisms are similar in the two cases. Using inhibitors of gap junctions and of voltage operated calcium channels, we showed that the locally induced calcium propagation likely depends on the propagation of the SMCs depolarization. Finally, from the experimental data gathered, it is possible to propose a model of the mechanisms underlying the propagation.

504-Pos
Edema: A Missing Link in the Conduction Velocity-Gap Junction Relationship
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Introduction: Myocardial edema i.e., increased extracellular volume (V_{ECS}) occurs in diverse pathologies. Often these states are also linked with sudden cardiac death and conduction slowing, sometimes linked to gap junction (Gj) remodeling. Yet, edema's effect on the conduction velocity (CV)-Gj relationship remains unknown. We hypothesized that edema modulates the CV-Gj relationship.
Methods: CV was quantified by optical mapping in Langendorff-perfused guinea pig ventricles while pacing from the anterior epicardium. Tissue water content was estimated by the wet weight (WW) to dry weight (DW; after 24h ≅ 60oC) ratio. V_{ECS} was modulated by perfusion of mannitol (26.1 g/l) or albumin (4 g/l). Gap junctions were uncoupled using carbenoxolone (10, 13 and 50 μM).
Results: Mannitol caused edema, increasing WW/DW ratio relative to control (10.7 ± 6 %, $p<0.05$, n=6). It decreased transverse CV (CV_T) by 24 ± 4% and longitudinal CV (CV_L) by 9 ± 2% ($p<0.05$, n=4). Consequently, edema increased anisotropy of conduction ($AR_{CV}= CV_L/CV_T$) from 2.30 ± 0.16 to 2.75 ± 0.19 ($p<0.05$). Albumin had the opposite effects. It dehydrated the heart, decreasing WW/DW relative to control (11.3 ± 5 %, $p<0.05$, n=6). Dehydration increased CV_T by 71 ± 10 % ($p<0.05$, n=4) without significantly affecting CV_L. Thus, dehydration reduced AR_{CV} to 1.84 ± 0.07 ($p<0.05$, n=4). Under control conditions, 10 and 13 μM carbenoxolone did not significantly affect CV while 50 μM carbenoxolone slowed CV_T by 25 ± 1 % ($p<0.05$, n=3) but had no effect on CV_L. During edema, 13 μM carbenoxolone significantly slowed CV_T by 38 ± 9 % ($p<0.05$, n=4) but had no effect on CV_L. Conduction was completely abolished by 50 μM carbenoxolone and mannitol.
Conclusions: These data demonstrate that edema slows conduction and unmasks a steeper CV-Gj relationship. Therefore, these data may explain conduction differences between nearly identical models of Cx43 down-regulation. Specifically, V_{ECS} determines whether Gj uncoupling slows conduction.

505-Pos
Increased Intercellular Communication in Activated Cardiac Fibroblasts
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Rationale: A critical event in the development of cardiac fibrosis is the transformation of fibroblasts into myofibroblasts; however the electrophysiological consequences of this phenotypic switch remain largely unknown. **Hypothesis:** Fibroblast activation results in a distinct electrophysiological phenotype that makes the heart more susceptible to arrhythmias. **Methods:** Ventricular fibroblasts were isolated from the ventricles of normal (Fb) and infarcted (MI-Fb) adult rat hearts. Fb and MI-Fb were plated onto myocyte monolayers at densities of 200, 400 and 600 cells/mm^2. Cultures were optically mapped with a voltage sensitive dye and conduction velocity (CV) and action potential duration (APD_{70}) were obtained. Functional intercellular communication, Cx43 expression levels and distribution were determined. **Results:** At 200 cells/mm^2, MI-Fb significantly increased (22.0 ± 0.6cm/s) CV compared to homocellular myocyte cultures (Myo; 17.8 ± 0.4cm/s). Fb (145.0 ± 3.9ms) and MI-Fb (131.1 ± 3.7ms) significantly reduced APD_{70} compared to Myo (158.6 ± 2.5ms), and APD_{70} was significantly shorter with MI-Fb compared to Fb. At higher fibroblast densities CVs were slower compared to Myo (Fb: 13.8 ± 0.4 and 11.4 ± 0.3cm/s; MI-Fb: 10.6 ± 0.3 and 9.5 ± 0.3cm/s, at 400 and 600 cells/mm^2, respectively). Fb and MI-Fb CVs were significantly different at all plating densities. At higher densities APD_{70} was significantly longer for Fb compared to Myo (171.0 ± 2.1 and 165.7 ± 1.8ms at 400 and 600 cells/mm^2, respectively). MI-Fb values were significantly longer at 400 cells/mm^2 (175.8 ± 2.7ms). Cx43 staining was present in contact areas between myocytes and fibroblasts from both sources. Immunoblotting showed a significant increase of Cx43 levels in MI-Fb compared to Fb. Intercellular coupling evaluated with gap-FRAP was significantly increased between myocytes and MI-Fb compared to Fb. **Conclusions:** These data demonstrate fibroblast activation results in important electrophysiological changes that could contribute to the greater incidence of arrhythmias observed in fibrotic hearts. These observations highlight the fibroblast activation process as a potentially new therapeutic target for arrhythmia prevention.

506-Pos
Astrocytes Control Breathing Through pH-Dependent Vesicular Release of Atp
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Extracellular signalling mediated by ATP has been associated with central chemosensory function. It has been shown that increase in pCO2 in the arterial blood triggers an immediate release of ATP from the chemosensitive regions located on the ventral surface of the medulla oblongata (VMS).
Using in vitro and in vivo preparations and novel genetically encoded Ca2+ indicator based on cyclically permutated GFP - Case 12, we show that astrocytes in the ventral regions of the medulla oblongata are highly sensitive to changes in pH. Decrease in extracellular pH from 7.4 to 7.2 induced transient increases in [Ca2+]i in astrocytes from dissociated neuro-glial cultures prepared from the VMS, in ventral astrocytes of organotypic brainstem slice cultures and in acute horizontal brainstem slices of adult rats, as well as on the VMS in anaesthetized and artificially ventilated rats. ATP receptor antagonists such as MRS2179, PPADS and TNP-ATP effectively blocked [Ca2+]i responses evoked by lowering pH in VMS astrocytes. ATP hydrolyzing enzyme apyrase completely prevented propagation of [Ca2+]i excitation in VMS astrocytes evoked by lowering external pH. These data suggest that Ca2+ responses induced by lowering external pH are mediated by ATP release and subsequent activation of P2 receptors. Inhibitors of vesicular transport brefeldine A and bafilomycin A both effectively abolished [Ca2+]i excitation of VMS astrocytes evoked by lowering external pH. Incubation of astrocytes with FM 1-46 dye (vesicular marker) leads to selective labelling of intracellular vesicles. Acidification of external medium induced decrease in the fluorescence intensity of

vesicles labelled with FM 1-43. These data suggest that VMS astrocytes respond to a decrease in pH by releasing ATP via vesicular exocytosis.

507-Pos

The Role of Cell Adhesion Molecule 1 (CADM1) in Nerve-Mast Cell Communication

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It has long been demonstrated that the nervous and immune systems are not disparate entities. The nerve-mast cell relationship served as prototypic association, and unequivocal evidence has been considerably presented for the consistent anatomical association and the functional interaction between nerves and mast cells. We found that nerve-mast cell communication can occur bidirectionally in the absence of intermediary cells using in vitro coculture approach and calcium imaging analysis. We have studied the molecular mechanism in nerve-mast cell communication and showed that substance P was an important mediator from superior cervical ganglia (SCG) to mast cells and induced the degranulation to mast cells attached with SCG neurites. In addition, ATP released from antigen-stimulated mast cells was found to activate SCG neurites attached with mast cells. To investigate the adhesion molecules involved in the nerve-mast cell communication, we here focused an adhesion molecule of immunoglobulin superfamily, CADM1, which is expressed on bone marrow-derived mast cells from wild type mice. When mast cells with or without CADM1 were cocultured with SCG and dorsal root ganglia (DRG) neurons, the number of CADM1-expressing mast cells attached to neurites was much higher than CADM1-deficient cells. The transfection with CADM1 to CADM1-deficient mast cells recovered the attachment to neurites. The responding rate of mast cells with CADM1 attached to neurites following specific activation of neurons by scorpion venom was higher than ones without CADM1. Ectopic expression of CADM1 increased this proportion. CADM1 was also found to be locally concentrated at points of contact between neurites and mast cells. These results suggested that CADM1 on mast cells not only functions as simple glue in nerve-mast cell interaction but also promotes development of a microenvironment to communicate efficiently each other.

508-Pos

Tunneling Membrane Nanotubes Generate Local Calcium Signals and May Actively Propagate Calcium Signals Between Cells

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Cells have long been known to employ gap junctions and synapses to communicate with their neighbors. A new mechanism has recently been proposed following the discovery of tunneling membrane nanotubes (TNTs) between cells [1]. TNTs are dynamic membrane protrusions with lengths up to several tens of microns and diameters of 50-800nm, which permit the exchange of membrane components and cytoplasmic molecules between neighboring cells. Ca^{2+} diffusion along TNTs has been proposed as a means of intercellular communication [2], yet our modeling simulations show that passive diffusion alone is insufficient to account for efficient transmission of Ca^{2+} between cells. Instead, we observe local spontaneous and inositol trisphosphate (IP_3)-evoked mediated Ca^{2+} signals within the length of TNTs formed between cultured SHSY-5Y neuroblastoma cells. Moreover, immunostaining demonstrates the presence of both ER and IP_3 receptors along the TNT. We propose that IP_3Rs are involved in actively propagating intercellular Ca^{2+} signals along TNTs, acting as amplification sites to overcome limitations of passive diffusion in a chemical analog of electrical transmission of action potentials along axons.

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Epithelial Channels & Physiology

509-Pos

Effects of Kcne Subunit Deletion on Polarized Trafficking of the KCNQ1 Potassium Channel *in Vivo*

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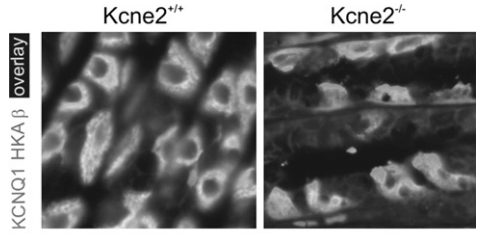
The KCNQ1 potassium channel alpha subunit generates essential K^+ currents in human heart and in a range of polarized secretory epithelia. The polarity of KCNQ1 trafficking varies between different epithelia, but neither the importance nor the mechanism for this polarity are well understood. KCNQ1 co-localizes apically with the KCNE2 beta subunit in gastric parietal cells but basolaterally with KCNE3 in colonic crypts. Both KCNE2 and KCNE3 convert KCNQ1 to a constitutively active channel. Here, genetic deletion of *Kcne2* in mice resulted in 5-fold upregulation of Kcne3, formation of Kcnq1-Kcne3 complexes, and basolateral Kcnq1 targeting in parietal cells, and gastritis cystica profunda stemming from achlorhydria and earlier hyperplasia. In contrast, *Kcne2*$^{-/-}$*Kcne3*$^{-/-}$ mice exhibited apical parietal cell Kcnq1 localization. Thus, in parietal cells, apical Kcnq1 localization is required for gastric acid secretion, and the apical localization *per se* does not require Kcne2. Kcne3, if present, actively targets Kcnq1 basolaterally, ultimately causing a preneoplastic condition which in humans could predispose to gastric cancer.



Apical (left) versus basolateral (right) localization of KCNQ1 (red) in gastric parietal cells of *Kcne2*$^{+/+}$ (left) versus *Kcne2*$^{-/-}$ (right) mice. Green: H^+/K^+-ATPase β subunit (apical marker).

510-Pos

Regulation of Delta-Enac Ion Channels by the Neuronal-Specific Sgk1.1 Kinase

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The epithelial sodium channel (ENaC) is a voltage-independent ion channel that plays a fundamental role in kidney transepithelial sodium transport and extracellular volume homeostasis. Previous work from our group and others have identified a novel ENaC subunit that is prominently expressed in neurons but not in kidney epithelia. The physiological role of delta-ENaC channels in neurons is unknown, but it could be involved in the regulation of membrane resting potential and hence of neuronal excitability. Kidney ENaC activity is increased by the serum and glucocorticoid-induced kinase 1 (SGK1). Recently, a new neuronal-specific isoform of SGK1, named SGK1.1, has been identified. We have tested whether SGK1.1 regulates delta-ENaC activity. Co-injection of SGK1.1 and delta-ENaC channels in *Xenopus* oocytes increased sodium current by two-fold. SGK1.1 increased delta-ENaC plasma membrane expression by 1.6-fold. *In situ* hybridization experiments confirmed the co-expression of delta ENaC and SGK1.1 in pyramidal neurons of the human cerebral cortex, indicating that this regulation could be physiologically relevant. In summary, we have identified a new regulator of delta-ENaC ion channels that could play a role in the control of neuronal resting potential and excitability.

511-Pos

A Multidomain Model For Electrodiffusion and Water Flow

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Fluid flow and its coupling to electrodiffusion is involved in many physiological systems from the kidney to the lens of the eye, where it has been studied in some detail (Journal of Membrane Biology (2007) 216:1-16). We formulate a mathematical model that describes electrodiffusion and water flow in three dimensions with resolution and scale appropriate for analysis of tissues. The mathematical model presented can be seen as a coarse-grained version of a model used in (PNAS(2008) 105:6463-6468) to model cellular and subcellular electrodiffusion. We shall discuss the relationship of the general model to other macroscopic models in electrophysiology, and show preliminary computations and applications.

Calcium Signaling Pathways

512-Pos

Orai3 and the Selective Activation of the Arc Channel by Arachidonic Acid

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